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DETECTION OF "HONEYDEW" ON RAW COTTON

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QUALITATIVE TEST FOR HONEYDEW[2]

Apparatus

1. Source of longer wavelength ultraviolet radiation: G.E. Purple-X bulb (250 watts, 115-125 volts) in a suitable reflector is satisfactory. Use in a darkened room.
2. Test tubes, Pyrex; approximately 18 x 150 mm.
3. Glass rods; approximately 5 x 200 mm.
4. Glass funnels; approximately 35 mm. diameter.
5. Cylinders, graduated; 10-ml. capacity.
6. Filter paper, 5.5 cm. diameter; Whatman No. 4, Carl Schleicher and Schuell No. 604, or a similar paper.
7. Balance, capable of weighing small amounts of substances with reasonable accuracy, with gram or grain scales.

Reagents

1. Distilled water.
2. Alcoholic alpha-naphthol, 10% solution. Dissolve 1.0 gram (15.4 grains) of alpha-naphthol in 10 ml. of 95% ethyl alcohol (U.S.P.). Store in a brown dropping bottle. Do not use if more than one week old.
3. Sulfuric acid, concentrated. Reagent grade.

Procedure

Select representative portions of the cotton suspected of being contaminated with honeydew. (These portions may be stained greenish-yellow and give a reddish fluorescence of low intensity when examined under ultraviolet light; or they may be molded due to growth of mold on the honeydew; or the areas may be sticky or crusty.) Weigh approximately 0.2 gram (or 3.1 grains) of the contaminated areas and transfer to a test tube. Select and weigh approximately 0.2 gram (or 3.1 grains) of clean, uncontaminated cotton from the same sample to be used as a control, and transfer to a similar tube.

To each tube add 2 ml. of distilled water from a graduated cylinder. Macerate each sample thoroughly for over 10 minutes with a glass rod, making certain that the sample is thoroughly wetted.

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[1] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

[2] If the glassware and reagents required are not available locally, they may be obtained from a laboratory supply company.

Fold 2 circles of filter paper, place each in a funnel, wet the papers with distilled water, and shake out excess water. Place a test tube under each funnel. Squeeze the extract from each cotton sample with the fingers (wash the fingers before squeezing each sample) and filter through the filter papers into the test tubes.

Measure from 0.7 to 1 ml. of each filtered extract and transfer to clean test tubes. (Use the same volume of each extract.) Add 4 drops of 10% alcoholic alpha-naphthol solution, followed by 8 ml. of concentrated sulfuric acid, to each tube. Stir the contents of each tube with a glass rod. Note the color of each solution.

The extracts from honeydew-contaminated areas give dark wine red or purple red colors, whereas the extracts from the control areas usually give pale pink or pale violet colors.

A small drop of corn sirup or strained honey may be added to water and tested to familiarize one with the color developed on reaction with the alpha-naphthol under the conditions of the test as specified.

Caution: The glass apparatus used in performing the test must be thoroughly washed and then rinsed with distilled water after each use. It is important that no cotton lint or filter paper fibers be present in the solution finally tested as these materials consist primarily of cellulose which will give a positive test.

NATURE AND OCCURRENCE OF HONEYDEW

Market lots of cotton are encountered frequently which are contaminated with a sticky or gummy substance making the cotton difficult to process in the mill. This substance is "honeydew." It is the excreta of aphids resulting from the partial digestion of plant sap on which these insects feed. Honeydew usually contains reducing sugars, sucrose, dextrin, and other carbohydrates (1), varying in amounts according to the nature of the sap used as food by the aphids. The analyses of honeydews from 4 kinds of aphids have been reported (4) as follows:

	A	B	C	D
Glucose	25.4%	14.3%	51.5%	43.7%
Fructose	13.9	0.0	0.0	--
Sucrose	36.2	35.8	11.1	39.9
Dextrin	11.1	28.2	0.0	11.1

The cotton aphid (aphis gossypii Glover), a small soft-bodied insect varying in color from pale yellowish green to almost black, is the principal aphid causing damage to the cotton plant by sucking sap and interfering with leaf function (2,3,5). Severe aphid infestations may occur in any area where cotton is grown and may follow excessive dusting of cotton with calcium arsenate for control of boll weevil. Dusting seems to favor the multiplication of aphids. The aphid excreta, honeydew, may cover the upper surfaces of the leaves and bolls of the cotton plant. If it falls on the lint while the bolls are opening or afterwards, the lint may become stained and be gummy. As honeydew is

hygroscopic and is composed largely of water-soluble substances, it may spread over a larger amount of lint than was originally contaminated. This would be especially true should the lint in the unpicked and opened boll be dampened by rain or dew. The honeydew may be consumed as nutrients for molds and the surface properties of the lint altered to an extent that it may be difficult to process, even though the presence of honeydew may not be detectable.

Honeydew usually gives a reddish fluorescence of low intensity when examined under ultraviolet light. This test is not conclusive, since a similar fluorescence is exhibited by cottonseed hulls, leaf particles, and portions of insects that may be present in cotton lint samples. However, the fluorescence can be used as an aid in distinguishing and selecting the most heavily contaminated portions of the lint sample on which a reliable confirmatory qualitative test may be applied.

For those needing to examine cotton for the presence of honeydew, the qualitative test described above is suggested.

APPLICATION OF TEST

Since uncontaminated raw cotton contains small amounts of sugars which are partially extracted under the conditions of the suggested test, it is desirable to run a control test on uncontaminated portions along with contaminated portions from the same sample of cotton lint. The control gives but a slight coloration, whereas the portions contaminated with honeydew should give a strong coloration. Although the honeydew-contaminated portions of the lint usually can be selected for the test because of their stained, sticky, or moldy appearance, ultraviolet fluorescence is especially helpful in the case of many samples.

The lint of 7 samples of seed cotton known to be contaminated with honeydew was examined by use of the suggested qualitative test. In addition, the reducing sugars, calculated as glucose, were determined quantitatively. The results are tabulated in table I. It is evident that the intensity of the color developed in the test is related to the percentage of reducing sugars present. Samples 1 and 2 giving the strongest test contained 0.48 and 0.40% reducing sugars, respectively. Normal raw cotton lints contain from 0.02 to 0.20% reducing sugars, generally less than 0.1%. Samples 3 to 7, inclusive, were picked after exposure to rain and had considerable mold on the portions originally contaminated with honeydew. In general, these 5 samples gave tests of slight intensity and were low in reducing sugars. Both factors, leaching by rain and mold growth, apparently were responsible for the greatly reduced content of reducing sugars.

The techniques specified for use in the suggested qualitative test have been successfully applied by several individuals without chemical training and experience.

For those desiring more details regarding aphids and honeydew with some reference to cotton, the articles cited below should prove informative.

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Table I. Reducing Sugar Contents of Honeydew-Contaminated Cottons and Results of Qualitative Tests for Honeydew in These Cottons

Sample No.	Variety and Description[1]	Reducing Sugars (calcd. as glucose)	Test for "Honeydew"
		%	Intensity of color
1	Acala 4-42, picked before rain.	0.48	Very strong
2	Acala 4-42, picked before rain.	0.40	Strong
3	Same as 1, except picked after exposure to rain.	0.027	Slight
4	Acala P 18-C, picked after exposure to rain.	0.004	Slight
5	Acala Youngs 28, picked after exposure to rain.	0.006	Moderate
6	AHA 4-1-108-48, picked after exposure to rain.	0.003	Slight
7	Acala 1517 W, picked after exposure to rain.	0.012	Slight

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[1] From delayed pickings at U. S. Cotton Field Station, Shafter, California.

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